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Stereochemistry and Active Conformation of a Novel Insecticide, Acetamiprid*

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Abstract: Acetamiprid, (NI-25; (*E*)-N^1-[(6-chloro-3-pyridyl)methyl]-N^2-cyano-N^1-methylacetamidine), is a novel neonicotinoid insecticide having an *N*-cyanoacetamidine structure as its characteristic feature. The [^{1}H] and [^{13}C]-NMR spectra indicated the existence of two different structures in acetamiprid at room temperature in solution. The measurement of CH-NOE and C–C coupling constants proved the *E*-configuration at the cyanoimino group in both existing structures. The [^{13}C] chemical shifts of *N*-CH_3 and the long range C–H coupling in the formamidine analogue of acetamiprid suggested that there exist two conformers generated by the rotation of C–C single bond in the amidine moiety. Dynamic NMR spectra of acetamiprid and the computer simulation of the two-site exchange demonstrated that the two conformers change slowly to each other at room temperature. The conformational analysis by semi-empirical molecular orbital calculations using MNDO-PM3 method predicted four conformers as energy-minimum structures, among which two *E*-conformers were more stable than *Z*-conformers. One of the *E*-conformers in which two methyl groups are in *cis* configuration was superimposable onto the structure of imidacloprid, which is a known neonicotinoid insecticide having more rigid structure. This *E*-conformer was assumed as the active conformation of acetamiprid on the basis of the molecular similarity in terms of steric and electrostatic properties.

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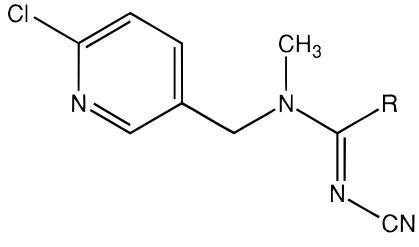
1 INTRODUCTION

Acetamiprid, (*E*)-N^1-[(6-chloro-3-pyridyl)methyl]-N^2-cyano-N^1-methylacetamidine (Fig. 1, **1**), is a novel insecticide developed by Nippon Soda Co., Ltd to control various noxious insects in agriculture.[1] Mode-of-action studies of acetamiprid suggested that the nicotinic acetylcholine receptor (nAChR) is its primary binding site, as with neonicotinoid insecticides such as imidacloprid.[2] The *N*-cyanoacetamidine structure of acetamiprid is unique and characteristic since most known neonicotinoids have nitromethylene or nitroimine structures. There are two isomeric forms in acetamiprid i.e. *E*- and *Z*-configurations of the cyanoimino group, and a number of stable conformers are expected to exist with respect to the rotation of single bonds in the *N*-pyridylmethylamino group. In order to predict the



1 (R = CH_3) : acetamiprid
2 (R = H)

Fig. 1. Structure of acetamiprid (**1**) and its formamidine analogue (**2**) used in this study.

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stable and existing structures of acetamiprid, we have attempted to investigate the stereochemical behaviour by using the techniques of NMR and computer-aided molecular modeling. Among a couple of stable conformers, an *E*-conformer was assumed as the active conformation of acetamiprid based on the aspects of molecular similarity between acetamiprid and the known neonicotinoid insecticide, imidacloprid.

2 MATERIALS AND METHODS

2.1 Compounds

Acetamiprid (**1**) and its formamidine analogue (**2**) (Fig. 1) synthesized in our research centre were used in the NMR studies. The synthetic methods used for them are described in the patent of this series of compounds.[3]

2.2 NMR analysis

[^{1}H] and [^{13}C]NMR spectra were recorded on a JOEL GSX-400 spectrometer at 400 MHz in deuterochloroform or hexadeuterodimethylsulfoxide (99% deuterized) as solvents. The referential standard for measuring resonance shifts was tetramethylsilane as an internal standard. Chromium(III) acetylacetonate[4] was used as a paramagnetic relaxation reagent in the measurement of NOE spectra.

The simulation of dynamic NMR for the two-site exchange of acetamiprid was performed by line-shape analysis.[5] The computer program of line-shape analysis, SITE2 (Gerig, J. T., pers. comm.), was employed and slightly modified to run on a NEC PC-9801 personal computer.

2.3 Conformational analysis and molecular electrostatic potentials

The conformational analysis of acetamiprid was performed by semi-empirical molecular orbital calculations using the MNDO-PM3 method[6,7] with MOPAC program.[8] To construct initial structures of acetamiprid, we have considered configurations and conformations of the molecule as follows:

(i) *E* and *Z* configurations of the cyanoimino group,
(ii) *cis* and *trans* orientations of the two methyl groups for the rotation of a C–N single bond in the acetamidine moiety,
(iii) six conformers for the rotation of an N–CH_2 bond by 60° steps representing *trans* and *gauche* structures,
(iv) rotation of the pyridine ring ($\pm$90° as the initial torsional angle).

Thus the 48 conformers were constructed and subjected to geometry optimization by MNDO-PM3 calculations.

The molecular electrostatic potentials of acetamiprid and related compounds were computed from the atomic charges derived from electrostatic potentials[9] by using the 'ESP' option in MOPAC.

2.4 Molecular similarity indices

As quantitative measures of molecular similarity, a method of similarity indices proposed by Richards and Hodgkin[10] was applied to study the electrostatic and shape similarity between acetamiprid and a known neonicotinoid insecticide, imidacloprid. The electrostatic similarity index (R_{AB}) is defined by eqn (1),[11] where ε_A and ε_B are electrostatic potentials of molecules A and B, respectively, at a point outside the two molecules superimposed. The value of the index varies in the range of -1 to 1, with $R_{AB} = 1$ indicating perfect similarity. The shape similarity index (S_{AB}) is defined in same manner by eqn (2),[12] where T_A and T_B are the volumes of the individual molecules A and B, respectively, and C is the volume commonly shared by the two molecules at the superimposition. The product of R_{AB} and S_{AB} (eqn (3)) is an alternative index defined by ourselves to evaluate both electrostatic and shape similarities by a single index.[13] The optimum superimposition of two molecules to maximize the similarity indices was obtained by simplex optimization procedure.[14]

$$R_{AB} = \frac{\int \varepsilon_A \varepsilon_B \, d\tau}{\left(\int \varepsilon_A^2 \, d\tau \int \varepsilon_B^2 \, d\tau\right)^{1/2}} \quad (-1 \leqq R_{AB} \leqq 1) \qquad (1)$$

$$S_{AB} = C/(T_A T_B)^{1/2} \qquad (0 \leqq S_{AB} \leqq 1) \qquad (2)$$

$$RS_{AB} = R_{AB} S_{AB} \qquad (-1 \leqq RS_{AB} \leqq 1) \qquad (3)$$

In the calculation of the similarity indices, R_{AB} and RS_{AB}, the values of ε_A and ε_B were calculated at each grid point of 0·3 Å intervals within the distance of 3·0 Å from the edge of the van der Waals surfaces of each molecule, and the space within the van der Waals surfaces of the two molecules was excluded from the calculation. The computer programs to calculate the similarity indices and to obtain the optimum superimposition were written on SGI IRIS-4D workstation.

3 RESULTS AND DISCUSSION

3.1 [^{1}H] and [^{13}C]NMR spectra

The [^{1}H] and [^{13}C]NMR spectra of acetamiprid at room temperature are shown in Fig. 2, indicating that every proton and carbon of the molecule gave two individual signals. This suggests that acetamiprid is in two different structures in solution. In order to clarify the

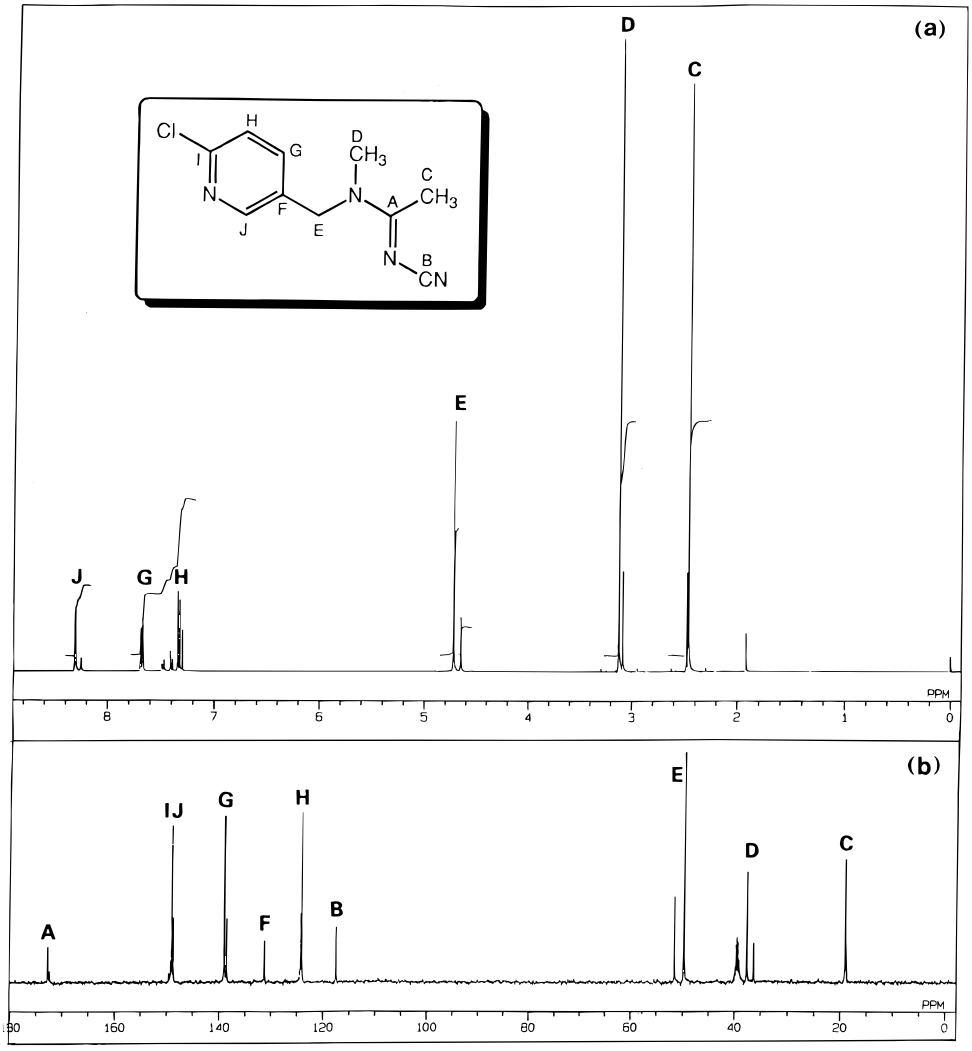


Fig. 2. NMR spectra of acetamiprid at 25°C. Assignment of each signal is shown by the corresponding letters in the chemical structure. (a) [^{1}H]NMR in deuterochloroform; (b) [^{13}C]NMR in hexadeuterodimethylsulfoxide.

structures of these two conformers, we have investigated the structural analysis of acetamiprid by means of NMR techniques.

In the measurement of CH-NOE spectra under various conditions, a significant CH-NOE signal was observed at the cyano carbon, when irradiating the CH_3 proton of the acetamidine group in the presence of chromium(III) acetylacetonate as a paramagnetic relaxation reagent at 100°C (Fig. 3). It was hence suggested that at least one of the conformers was in *E*-configuration where the irradiated methyl and the cyano group were in *cis* orientation. The coupling constants ($^1J_{CC}$) between the imino and CH_3 carbons of the acetamidine group were also measured by the 1D-INADEQUATE spectrum. The $^1J_{CC}$ values for the signals of both conformers were exactly the same (45·5 Hz). It has, however, been reported that the corresponding $^1J_{CC}$ values of *E*- and *Z*-oximes are not the same, i.e. the difference is *c.* 8–10 Hz,[15] because of the effect of the lone pair at the imino nitrogen. The identical $^1J_{CC}$ value for the two conformers of acetamiprid indicates that the configuration of the cyanoimino group is the same in both conformers whether it is *E* or *Z*. Thus the results of the CH-NOE spectrum and the C–C coupling constants in the *N*-cyanoacetamidine moiety support the *E*-configuration for both conformers of acetamiprid, where the cyano and methyl groups are in *cis* configuration.

To elucidate the structural difference in the two conformers, an analogous compound of acetamiprid having a formamidine structure (**2**) was subjected to NMR analysis. The chemical shifts of the *N*–CH_3 carbon and the long-range C–H coupling constants ($^3J_{CH}$) with the formamidine proton are shown in Fig. 4. We have compared the chemical shifts and the $^3J_{CH}$ values with those of *N*,*N*-dimethylformamide (DMF), since the assignment of the NMR spectra for the nonequivalent *N*-methyl groups in DMF has been described in the literature.[16] In DMF, the *N*-methyl carbon in *cis* orientation to the formyl proton gives a signal at 5·5 ppm lower field than the methyl carbon in *trans* at room temperature, and its coupling constant with the formyl

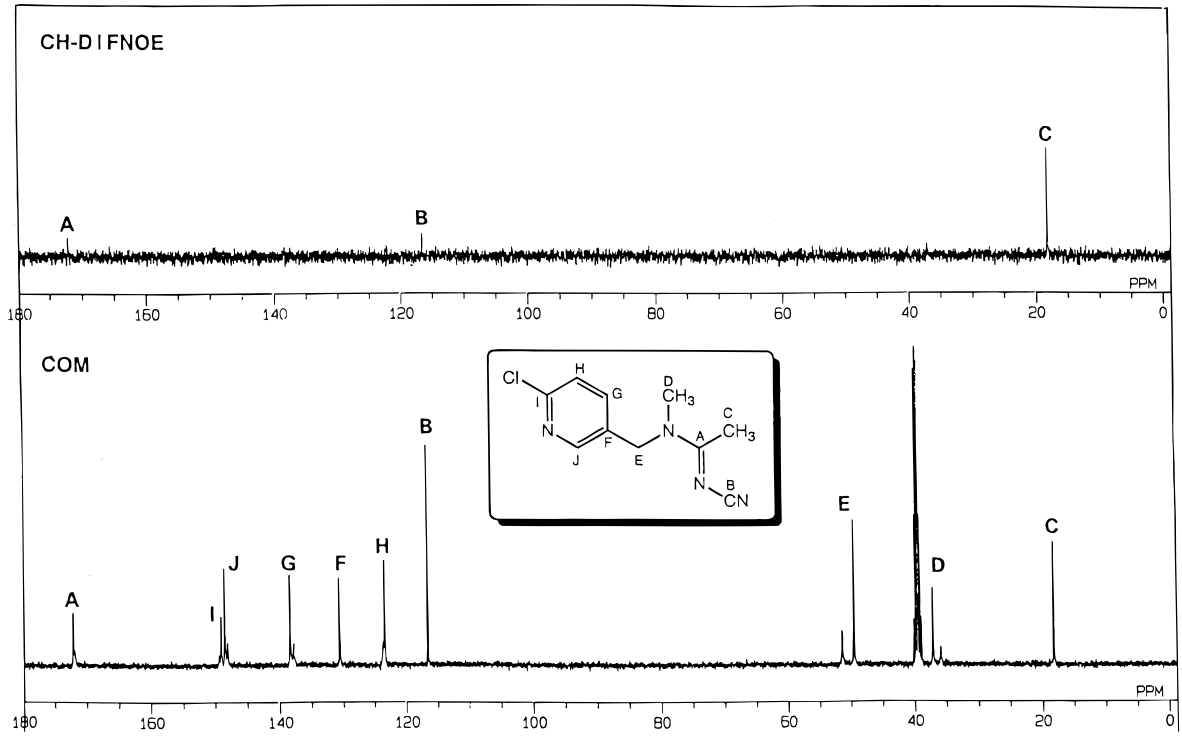


Fig. 3. [^{13}C]NMR spectrum of acetamiprid in hexadeuterodimethylsulfoxide at 100°C with chromium(III) acetylacetonate (below), and CH-NOE differential spectrum by irradiating the CH_3 proton (**C**) (above). The letters indicate the assignment of each signal in the chemical structure.

proton is 1·4 Hz smaller than that of the *trans* methyl. Assuming the similar character of the *N*-methyl carbon in compound **2**, the signal in lower field (38·9 ppm) giving smaller $^3J_{CH}$ (3·2 Hz) was assigned to a conformer in which the methyl group was *cis* to the formamidine proton, and the other signal in higher field (32·7 ppm) to the *trans* conformer where the methyl group was on the opposite side.

Based on the results of NMR analyses shown above, it is concluded that the two conformers of acetamiprid detected by NMR spectra are *cis* and *trans* conformers with respect to the conformation of the *N*-methylamino group, and that the *N*-cyanoimino group in both conformers is in *E*-configuration.

3.2 Dynamic NMR

Dynamic NMR of acetamiprid in hexadeuterodimethylsulfoxide was examined by changing temperature (Fig. 5). The methylene proton gave two signals at 30°C, and they fused into one peak at a higher temperature of around 90°C. The computer simulation of the site-exchange was also performed by the method of line-shape analysis, where the correlation

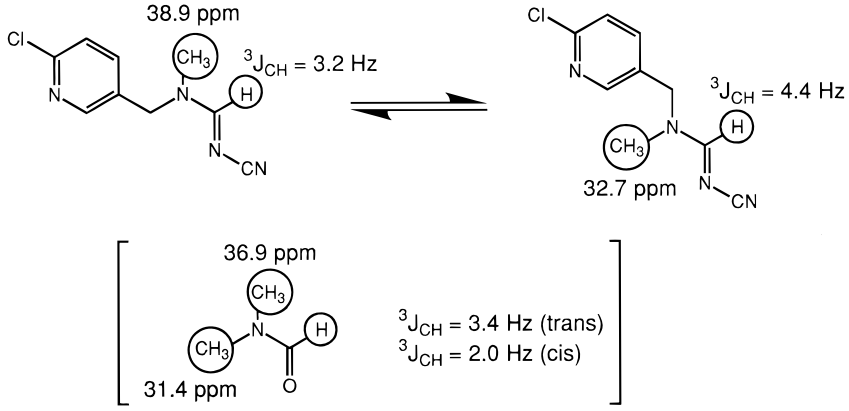


Fig. 4. [^{13}C] Chemical shifts of N–CH_3 in compound **2** and the long-range C–H coupling constants ($^3J_{CH}$) between the N–CH_3 carbon and the formamidine proton. The chemical shifts and $^3J_{CH}$ of N–CH_3 carbon in *N*,*N*-dimethylformamide are shown as a reference.

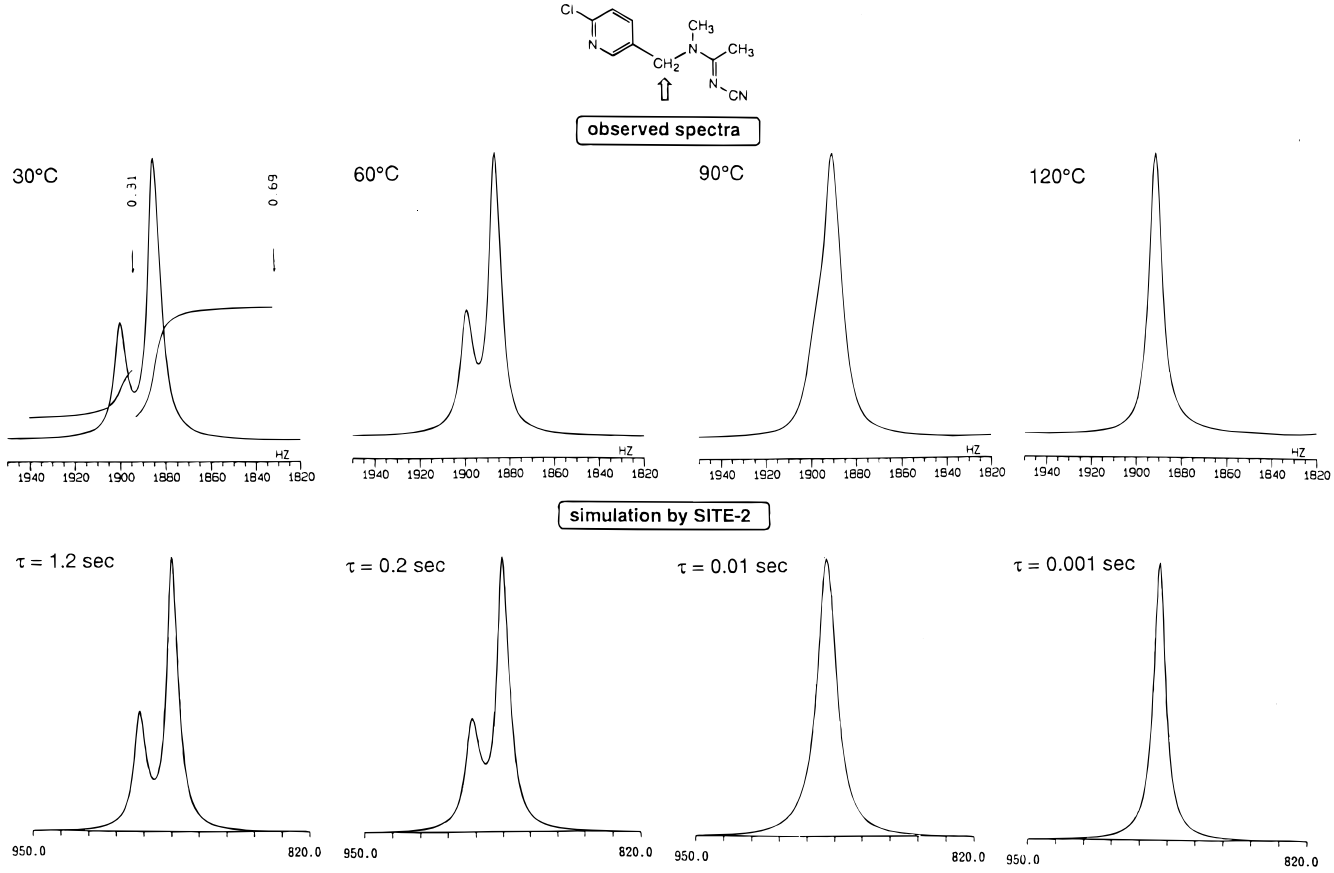


Fig. 5. The site-exchange of acetamiprid observed by NMR of the CH_2 proton and its computer simulation by line-shape analysis. The correlation time (τ) of the major component at each line-shape is shown in the simulated spectra.

time (τ) was varied to reproduce the observed spectra. The obtained theoretical spectra are compared with the observed ones in Fig. 5. The τ values of the two sites are 1·2 and 2·7 s, respectively, in the simulation of the spectrum at 30°C, suggesting that the two conformers change slowly into each other at room temperature. The two-sites exchange at higher temperature was simulated by giving smaller τ values at which faster exchange was expected.

3.3 Conformational analysis

The conformational analysis of acetamiprid by MNDO-PM3 calculations was performed to predict its stable conformations. Considering the *E* and *Z* configurations at the cyanoimino group and the rotation of single bonds, the geometry optimization was carried out for the 48 conformers constructed as described above. Among the optimized structures, the four conformers shown in Fig. 6 were obtained as energy minimum structures. The conformers **E-1** and **E-2** are in *E*-configuration at the cyanoimino group, and the conformations with respect to their two methyl groups are *cis* and *trans*, respectively. The conformers **Z-1** and **Z-2** are the *Z*-isomers corresponding to **E-1** and **E-2**, respectively. The relative energy of these conformers in terms of their heat of formation indicated that the conformers in *E*-configuration (**E-1** and **E-2**) are more stable than the *Z*-isomers. The relative stability of the two *E*-isomers was shown to be almost the same. The rotation of the pyridine ring in each conformer was considered to be flexible for free rotation. Another set of four conformers where the pyridine ring was reversed from each conformer in Fig. 6 was also obtained, and the heat of formation of each reversed conformer was almost the same as the corresponding one in Fig. 6.

3.4 Slow exchange of two conformers

From the results of NMR and conformational analysis shown above, there exist two *E*-conformers (**E-1** and **E-2**) in acetamiprid which exchange slowly with one another at room temperature in solution (Fig. 7). The rotation about the C–N single bond in the acetamidine moiety may be restricted as was reported for the rotation of the C–N$(CH_3)_2$ bond in *N*,*N*-dimethylformamide,[17] so that the exchange between the two conformers is slow enough to be detected by NMR. The activation energy (E_a) in this exchange was estimated from the dynamic NMR analysis by Arrhenius plot to

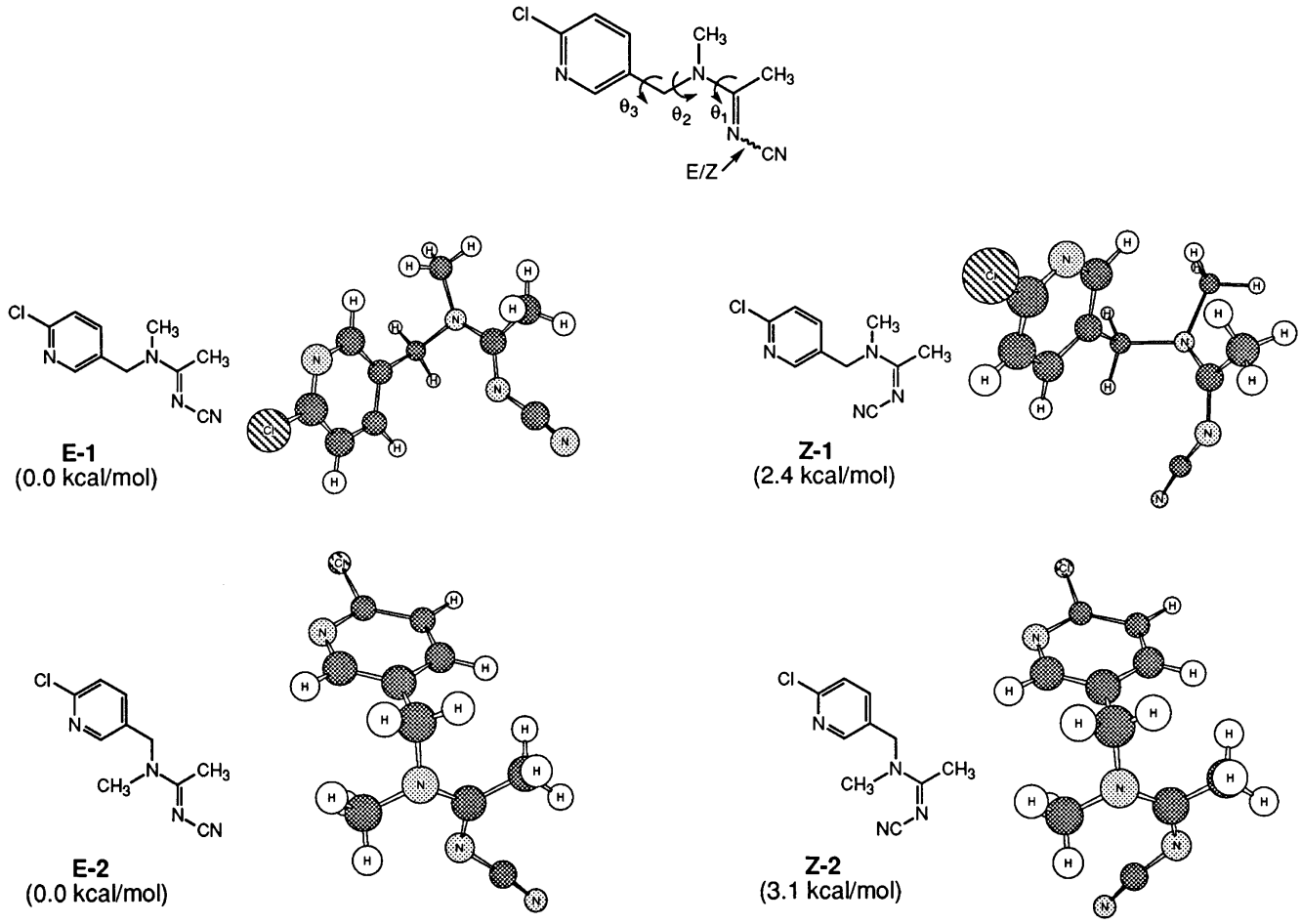


Fig. 6. Stable conformers of acetamiprid and their relative energy by MNDO-PM3.

be 19·0(±2·0) kcal mol^{-1} ($r = 0{\cdot}99$) which is comparable to the reported values[17] for *N*,*N*-dimethylformamide (20·5(±0·2) kcal mol^{-1} in carbon tetrachloride) and *N*,*N*-dimethylacetamide (21·3(±0·6) kcal mol^{-1} in formamide).

3.5 Prediction of active conformation

In order to predict the active conformation of acetamiprid, we have focused our attention on the molecular similarity in neonicotinoid insecticides. We have examined the steric and electrostatic similarities between acetamiprid and a known neonicotinoid, imidacloprid (**3**),[18] which has a rigid ring structure and a nitroimino group, and shows the same mode of action as acetamiprid (Fig. 8).

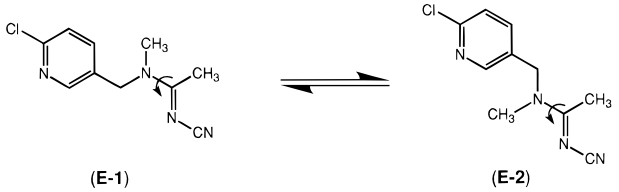


Fig. 7. Slow exchange of two stable conformers in acetamiprid.

3.5.1 *Molecular electrostatic potentials*

The molecular electrostatic potentials of the *N*-cyanoacetamidine moiety in acetamiprid were computed from the electrostatic-potential (ESP) derived atomic charges obtained by MNDO-PM3 method. The contour representations of the potentials were compared with the one calculated for the imidazolidine moiety in imidacloprid (Fig. 9). The negative contours are distributed around the cyano and nitro groups, and the positive potentials are in the opposite side, so that the patterns in potential maps of the two molecules are similar to each other. This suggests that the polar groups in acetamiprid and imidacloprid play a similar role in the electrostatic interaction at the receptor.

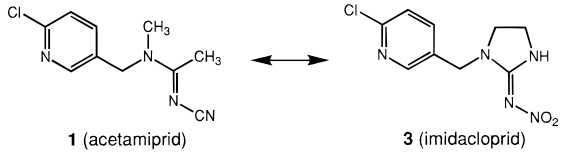


Fig. 8. Structures of acetamiprid (**1**) and imidacloprid (**3**) used in the molecular similarity analysis.

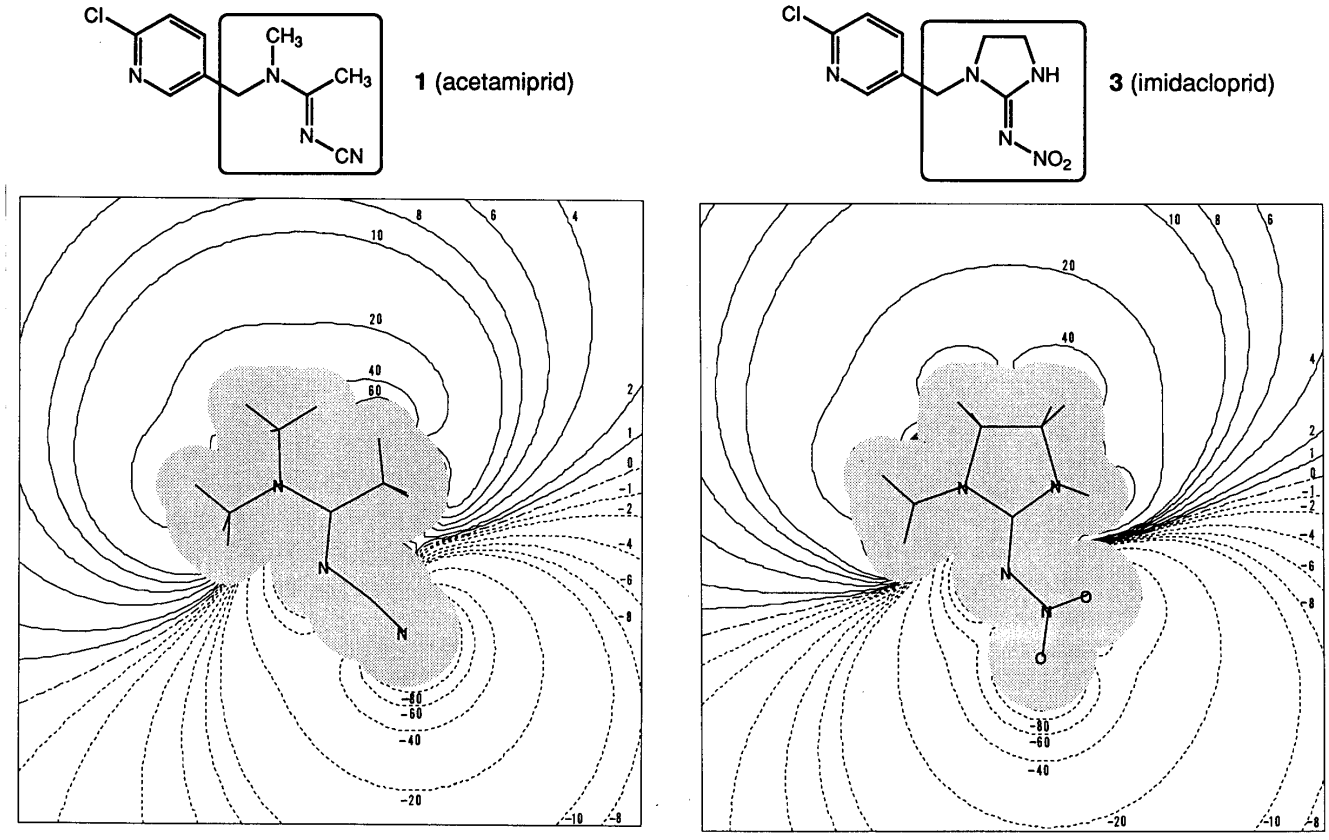
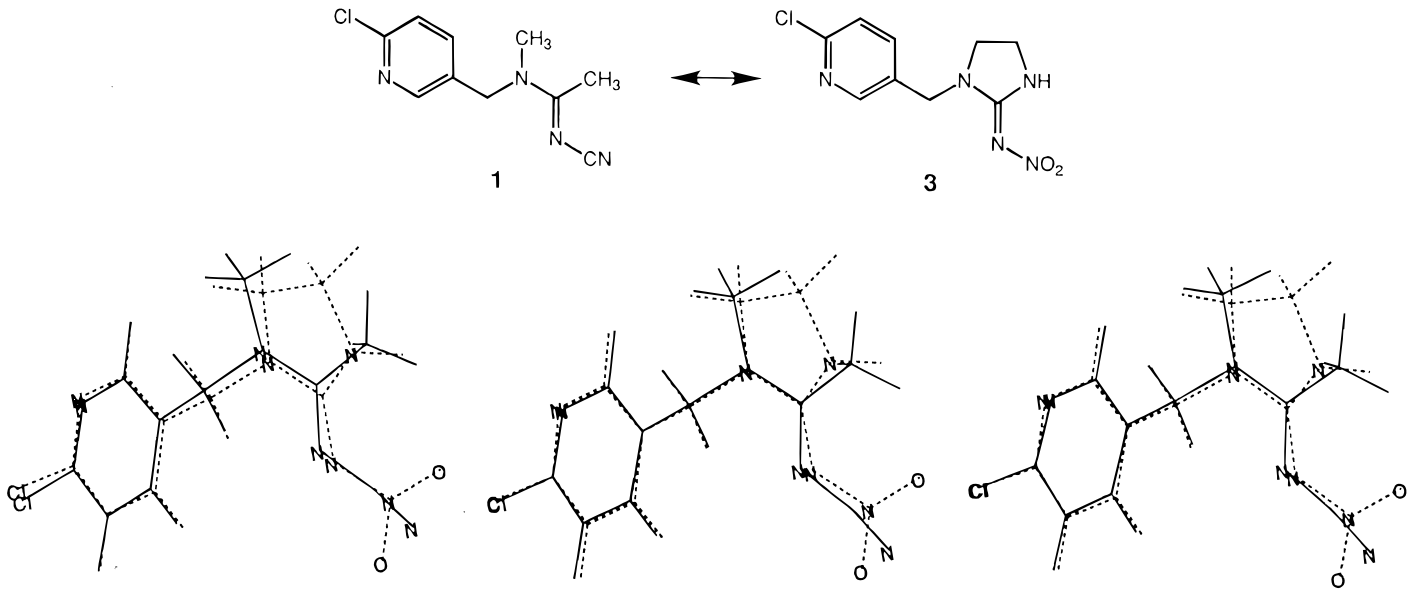


Fig. 9. Molecular electrostatic potentials of acetamiprid (left) and imidacloprid (right). Contour values are in unit of 10^{-3} au.



	R_{AB} optimized	S_{AB} optimized	RS_{AB} optimized
R_{AB}	0.940	0.928	0.933
S_{AB}	0.851	0.905	0.902
RS_{AB}	0.800	0.840	0.841

Fig. 10. Superimposed models of acetamiprid and imidacloprid to maximize the similarity indices (solid lines: acetamiprid, broken lines: imidacloprid). The values of similarity indices at each superimposition are shown below the models.

3.5.2 Superimposition and similarity indices

The *E*-configuration of the nitroimino group in imidacloprid has been confirmed by X-ray crystallography,[19] and our MNDO-PM3 calculations also supported that the *E*-form is more stable than the *Z*-form. We have examined the superimposition of acetamiprid onto the *E*-isomer of imidacloprid, and found that the conformer **E-1** is closely superimposed on imidacloprid. The optimum superimpositions were computed so as to maximize the similarity indices by simplex optimization procedure. In the superimposed models obtained to maximize each index, the relative orientations of the two molecules were almost the same (Fig. 10). In other words, the optimum superimposition to maximize the electrostatic similarity also satisfies the molecular shape similarity. The values of similarity indices for each superimposed models are shown in Fig. 10, indicating high molecular similarity in terms of both steric and electrostatic properties.

Based on the aspects of the superimposition study of acetamiprid onto imidacloprid, we have assumed the conformer **E-1**, one of the stable conformers, as the active conformation of acetamiprid.

4 CONCLUSION

Stereochemical behaviour of acetamiprid was studied by NMR and computer-aided techniques. It was revealed that there exist two *E*-conformers which exchange slowly with one another in solution. One of the *E*-conformers was found to be similar to imidacloprid in terms of steric and electrostatic properties, so that the active conformation of acetamiprid was assumed as the conformer **E-1**. Such similarities may play an important role for the molecular recognition of the bioisosteric species at the receptor. The method of similarity index was shown to be helpful in understanding the bioisosterism, since it provides a numerical measure of molecular similarity and optimum superimposed models of molecular structures to be compared. The quantitative correlation between the molecular similarity and the biological activity of neonicotinoid insecticides is under investigation, and will be presented in a paper following this study.

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